

## *Trichinella nativa* in a black bear from Plymouth, New Hampshire

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### Abstract

A suspected case of trichinellosis was identified in a single patient by the New Hampshire Public Health Laboratories in Concord, NH. The patient was thought to have become infected by consumption of muscle larvae (ML) in undercooked meat from a black bear killed in Plymouth, NH in October 2003 and stored frozen at  $-20^{\circ}\text{C}$  for 4 months. In January 2004, a 600 g sample of the meat was thawed at  $4^{\circ}\text{C}$ , digested in hydrochloric acid and pepsin, and larvae were collected by sedimentation. Intact, coiled, and motile ML were recovered (366 larvae per gram (lpg) of tissue), which were passed into mice and pigs. Multiplex PCR revealed a single 127 bp amplicon, indicative of *Trichinella nativa*. The Reproductive Capacity Index (RCI) for the *T. nativa*-Plymouth isolate in mice was 24.3. Worm burdens in the diaphragms of two 3-month-old pigs given 2500 ML were 0.05 and 0.2 lpg by 35 days post-inoculation, while 2.2 and 0.75 lpg were recovered from two 3-month-old pigs given 10,000 ML; no larvae were recovered from four 1-year-old pigs given 2500 ML ( $n = 2$ ) or 10,000 ML ( $n = 2$ ). Viable larvae were also recovered from frozen black bear meat harvested at two additional locations, one in southern Ontario, Canada, and one in upstate New York, USA. Multiplex PCR using genomic DNA from these parasite samples demonstrated that both isolates were *T. nativa*. This is the first report of the freeze-resistant species, *T. nativa*, within the continental United States. Published by Elsevier B.V.

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### 1. Introduction

Sylvatic isolates of the genus *Trichinella* are widespread in the environment due to an expansive host range and worldwide geographic distribution.

Though *Trichinella spiralis* is virtually absent from the U.S. pig population (NAHMS, 2000, unpublished), sylvatic isolates pose a risk for zoonotic transmission when wildlife has access to pig barns or non-confined animals. In addition, game animals serve as hosts for *Trichinella* species that can cause human disease if meats are not properly prepared.

Currently, eight sibling species and two genotypes of undetermined taxonomic status have been identified

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in the genus *Trichinella* (Kapel, 2000; Murrell et al., 2000; La Rosa et al., 2003). Geographic distribution of these isolates has been described (Pozio et al., 1992; Kapel, 1997; Pozio et al., 1998; Pozio, 2001a,b). Two of the sibling species, *Trichinella nativa* and *Trichinella* T6 have been identified as freeze tolerant and are capable of surviving in frozen muscle tissues for extended periods of time at temperatures from  $-5$  to  $-18$  °C (Kapel et al., 1999; Malakauskas and Kapel, 2003). *Trichinella* T6 has been identified in cougar and other carnivores from the Rocky Mountain region of the western continental United States, Pennsylvania, and Ontario, Canada (Dworkin et al., 1996). *T. nativa*, on the other hand, was thought to be confined to the arctic and subarctic zones of the Holarctic region (Murrell et al., 2000) and has not been previously reported within the continental United States or in areas where the January isotherm is higher than  $-5$  °C (Pozio et al., 1998). In this study, we describe an apparent southern geographic extension of the known range of *T. nativa* into the continental United States.

## 2. Materials and methods

Approximately 1.5 kg of frozen black bear meat was received at the Animal Parasitic Diseases Laboratory, Beltsville, MD, in December 2003. The bear meat had been frozen for at least 6 weeks and was kept frozen at  $-20$  °C until January 2004. Coiled intracellular larvae were noted on compression slides. Pepsin:HCl digestion (Gamble, 1996) of the bear meat revealed live, motile larvae which were washed by centrifugation. Five groups of 5 mice (Swiss-Webster females, Taconic Farms, Germantown, NY) were orally inoculated with 500, 1000, 1500, 2000, and 2500 muscle larvae (ML) each. After 21 days, larvae were recovered from skinned, eviscerated carcasses by pepsin:HCl digestion, enumerated, and photographed using an Olympus BX-2 digital photomicroscope.

The reproductive capacity index (RCI = #ML recovered/#ML inoculated) was determined in two groups of 10 mice inoculated with 50 or 100 ML and digested 35 days later as described above. The RCI index in pigs was determined by inoculating two groups of four pigs (Group 1: 1 year in age; Group 2: 3 months in age) with 2500 or 10,000 larvae. Thirty-five

days after inoculation, 100 g samples from tongue, diaphragm, loin, tail (50 g), shoulder, quadriceps, hams, shanks (front), masseter, intercostals, neck, tenderloin, flank, and throat were collected from each pig, weighed, digested, and worm burdens were determined. In addition, serum was collected from each pig at necropsy for ELISA (Gamble et al., 1997).

For multiplex PCR, ML recovered from the naturally infected bear meat, and experimentally infected mice and pigs, were each washed three times in Hanks Balanced Salt Solution, pelleted, and subjected to genomic DNA extraction utilizing a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA). The multiplex PCR was carried out essentially as described by Zarlenga et al. (1999).

During the same approximate time period, viable ML were obtained from two additional frozen black bear meat samples obtained (1) through a custom slaughterhouse in Clinton County, New York, and (2) from an animal shot and field dressed in Timmons, Ont., Canada, prior to transporting the meat to Tennessee. Multiplex PCR was carried out on DNA isolated from each parasite sample as described above.

## 3. Results

All mice survived the inoculation of 500–2500 larvae recovered from the New Hampshire bear meat. Infection efficiencies determined from mice inoculated with 50 or 100 ML resulted in mean larval worm burdens of 1260 ( $\pm 735$ ) and 2340 ( $\pm 1458$ ), respectively, and a mean RCI of 24.3. A single larva only was recovered from throat muscle of one pig in Group 1 (1 year of age) inoculated with 10,000 ML. No other larvae were recovered from Group 1 pigs. Viable ML were recovered from all four pigs in Group 2 (3 months of age) (Table 1). Pigs in Group 2 had a calculated worm burden of 0.05 and 0.2 lpg in animals inoculated with 2500 ML, and 2.2 and 0.75 lpg in animals inoculated with 10,000 ML. All pigs in both groups had high antibody titers to *T. nativa* E/S products in ELISA (Table 2).

Multiplex PCR revealed amplicons of the expected size (127 bp) for *T. nativa* in assays containing DNA from the naturally infected bear and from the experimentally infected pigs (Fig. 1). Multiplex PCR analysis of *Trichinella* isolates from both Clinton

Table 1  
Recovery of muscle larvae (ML) from 3-month-old pigs<sup>a</sup>

Muscle	2500 ML (pig 5/6)	10,000 ML (pig 7/8)
Diaphragm	10/40	220/74
Intercostal	0/40	58/38
Tail <sup>b</sup>	0/10	5/0
Shoulder	0/10	134/11
Tongue	10/50	216/53
Quadriceps	0/10	20/4
Ham	0/0	28/6
Shank-front	20/0	0/7
Mandibular	0/10	70/10
Neck	0/40	180/97
Loin	10/0	20/6
Tenderloin	0/40	140/62
Flank	1/30	96/28
Throat	0/0	70/25

<sup>a</sup> Total larvae recovered from 100 g muscle samples.

<sup>b</sup> Total larvae recovered from 50 g muscle samples.

County, NY, and Timmons, Ont., also revealed a single 127 bp amplicon, indicative of *T. nativa*. Muscle larvae were deposited with the International *Trichinella* Reference Center in Rome, Italy, and assigned the number ISS1552.

#### 4. Discussion

Based upon its resistance to freezing and the amplification of a single 127 bp target sequence in multiplex PCR assays, the isolate obtained from Plymouth, NH and described herein is *T. nativa*. The black bear, *Ursus americanus*, is territorial and will generally occupy an area usually 8–10 mile<sup>2</sup> (20–25 km<sup>2</sup>), though in less populated locations they may extend their range to over 15 mile<sup>2</sup> (40 km<sup>2</sup>). Thus, this limited range suggests that the animal taken in Plymouth, New Hampshire likely originated within

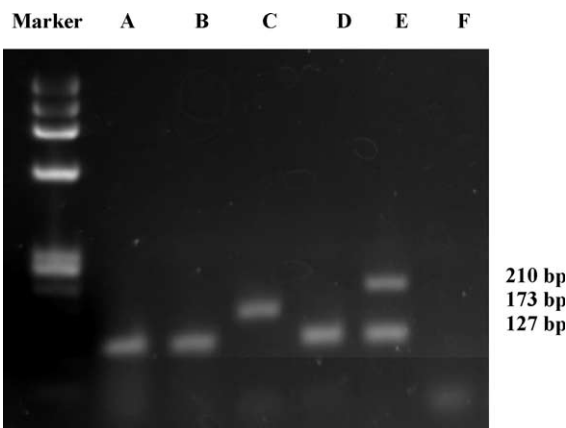


Fig. 1. Multiplex PCR for differentiating *Trichinella* genotypes (Zarlenga et al., 1999). (A) *T. nativa*-Plymouth isolate from experimentally infected pig, 127 bp; (B) *T. nativa*-Plymouth isolate from naturally infected black bear; (C) *T. spiralis* isolate ISS31 control; (D) *T. nativa* isolate ISS43 control; (E) *Trichinella* T6 isolate ISS46 control; (F) negative control.

United States boundaries making this the first report of *T. nativa* obtained from hosts living within the continental United States; however, aberrant migration patterns cannot be ruled out as the contributing factor. The very similar freeze tolerant genotype, *Trichinella* T6, has been identified in numerous geographical localities within North America including the United States; however, the multiplex PCR banding pattern clearly delineating the *Trichinella* T6 genotype was not observed in any of the analyzed samples. Previous studies have demonstrated *T. nativa* infections in a wide variety of mammalian hosts in the arctic and subarctic zones of the Holarctic region where the January isotherm is lower than  $-5^{\circ}\text{C}$  (Kapel, 1997; Pozio et al., 1998; Murrell et al., 2000) but never this far south in North America. Furthermore, although the outbreak in Tennessee did not

Table 2  
Serological responses and worm burdens in mice and pigs infected with *T. nativa*-Plymouth

Pig (number/age)	Number of muscle larvae given per os	ELISA titers	lpg
1 (1 year)	2500	0.920	0
2 (1 year)	2500	0.903	0
3 (1 year)	10000	0.922	0
4 (1 year)	10000	1.075	0
5 (3 month)	2500	1.102	0.05
6 (3 month)	2500	1.001	0.20
7 (3 month)	10000	0.998	2.2
8 (3 month)	10000	0.955	0.75

originate from a bear killed in the United States, it nonetheless demonstrates one possible method for dissemination of the freeze resistant genotype(s) to more temperate localities.

Previous studies by Kapel et al. (1998), and Kapel (2000) demonstrated that the infectivity level of *T. nativa* for domestic swine is below that which poses a public health risk (>1 lpg). In addition, these studies observed high serum antibody responses to *Trichinella* E/S antigens in all experimental groups, including pigs in which no muscle larvae were recovered. Similar antibody results were seen in the present study; however, recovery of viable ML above the level of public health significance was seen in one, 3-month-old pig given 10,000 ML. These data suggest that variability in exposure and resistance to *T. nativa* in domestic swine could result in pig infections with freeze tolerant species that could pose a public health risk, and that the level of pig resistance may be age related since higher infectivity rates were observed in younger animals (3 months of age) relative to older animals (1 year of age).

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